Municipality of Anchorage

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Mayor Dan Sullivan

Anchorage Water & Wastewater Utility

Board Chair Tim Sullivan

August 8, 2014

Director, Office of Water
U.S. Environmental Protection Agency, Region 10
NPDES Compliance Unit
1200 Sixth Avenue, OW-133
Seattle, Washington 98101

AUG 11 2014

Subject: **Whole Effluent Toxicity Testing Results**
2nd Quarter 2014
NPDES Permit No. AK-002255-1

The John M. Asplund Water Pollution Control Facility permit requires that quarterly whole effluent toxicity (WET) testing reports be submitted with the discharge monitoring report (DMR) for the month following the test month. The enclosed report outlines test results for the short-term chronic toxicity test conducted for the second quarter of 2014 (24-hour composite sample collected June 11th, 2014). Effluent flow on the sampling day for this WET test sample was 27.48 MGD.

The permit requires that testing continue with the most sensitive species after an annual screening using three species. This quarter's test used the most sensitive species as demonstrated by the screening of all three species in first quarter 2014. The WET testing consisted of a fertilization test using the purple sea urchin (*Strongylocentrotus purpuratus*). The permit toxicity trigger of 143TUc was not exceeded in this test with a reported chronic toxicity of 35.7 TUc.

> *"I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations."*

I can be contacted at (907) 564-2799 or mailto:David.Persinger@awwu.biz should you have any questions.

Sincerely,

David Persinger, P.E.
Director, Treatment Division - AWWU

Cc: Alaska Department of Environmental Conservation, Division of Water
Rob Gustafson, Water Quality Supervisor, AWWU
Jeff Axman, Acting Superintendent, John M. Asplund WPCF, AWWU

Enclosure: Pacific EcoRisk, WET test report

Community, Security, Prosperity

8/13/14

PACIFIC ECORISK ENVIRONMENTAL CONSULTING & TESTING

Gary Lawley
Kinnetic Laboratories, Inc.
1102 West 7th Avenue
Anchorage, AK 99501

July 11, 2014

Gary,

I have enclosed our report "NPDES Compliance Toxicity Testing of the City of Anchorage John M. Asplund Water Pollution Control Facility Effluent". This evaluation consisted of performing the US EPA echinoderm sperm fertilization short-term chronic toxicity test with the purple urchin, *Strongylocentrotus purpuratus,* using an effluent sample collected June 11, 2014. A summary of the results of this testing follows:

Chronic Effects of Anchorage Effluent on Purple Urchin Sperm Fertilization
There were ***no*** significant reductions in echinoderm sperm fertilization at the effluent concentrations tested; the NOEC was 2.8% effluent, resulting in 35.7 TUc.

If you have any questions regarding the performance and interpretation of this test, please contact my colleagues Dr. Scott Ogle or Alison Briden at (707) 207-7760.

Sincerely,

Stevi Vasquez
2014.07.14
09:46:53 -08'00'

Stevi Vasquez
Aquatic Ecotoxicologist

Pacific EcoRisk is accredited in accordance with NELAP (ORELAP ID 4043). Pacific EcoRisk certifies that the test results reported herein conform to the most current NELAP requirements for parameters for which accreditation is required and available. Any exceptions to NELAP requirements are noted, where applicable, in the body of the report. This report shall not be reproduced, except in full, without the written consent of Pacific EcoRisk. This testing was performed under Lab Order 22580.

NPDES Compliance Toxicity Testing of the City of Anchorage John M. Asplund Water Pollution Control Facility Effluent

Sample collected June 11, 2014

Performed For:

Kinnetic Laboratories, Inc.
1102 West 7th Avenue
Anchorage, AK 99501

Prepared By:

Pacific EcoRisk
2250 Cordelia Rd.
Fairfield, CA 94534

July 2014

PACIFIC ECORISK
ENVIRONMENTAL CONSULTING & TESTING

NPDES Compliance Toxicity Testing of the City of Anchorage John M. Asplund Water Pollution Control Facility Effluent

Sample collected June 11, 2014

Table of Contents

Appendices

1. INTRODUCTION

Kinnetic Laboratories, Inc., (Kinnetic) has contracted Pacific EcoRisk (PER) to perform an evaluation of the chronic toxicity of effluent collected from the City of Anchorage John M. Asplund Water Pollution Control Facility (Anchorage). This evaluation consisted of the US EPA echinoderm sperm fertilization short-term chronic toxicity test with the purple urchin, *Strongylocentrotus purpuratus*. This test was performed using an effluent sample that was collected on June 11, 2014. In order to assess the sensitivity of the test organisms to toxic stress, a reference toxicant test was also performed. This report describes the performance and results of these tests.

2. TOXICITY TEST PROCEDURES

The methods used in conducting this toxicity testing followed the guidelines established by the EPA manuals " Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms " (EPA/600/R-95/136).

2.1 Receipt and Handling of the Effluent Sample

On June 11, a sample of Anchorage effluent was collected into an appropriately cleaned sample container; this sample was shipped via overnight delivery, on ice and under chain-of-custody, to the PER testing facility in Fairfield, CA. Upon receipt at the testing laboratory, aliquots of the sample were collected for determination of initial water quality characteristics (Table 1), after which the remainder of the sample was stored at 0-6°C, except when being used to prepare the test solutions. The chain-of-custody record for the collection and delivery of the sample are provided in Appendix A.

Table 1. Initial water quality characteristics of the Anchorage effluent sample.

Sample Collection Date	Sample Receipt Date	Sample ID	Temp (°C)	pH	D.O (mg/L)	Salinity (ppt)	Conductivity (μS/cm)	Total Ammonia (mg/L N)
6/11/14	6/12/14	MOA14TOX004	1.7	7.24	7.5	0.4	726	25.1

2.2 Echinoderm Fertilization Toxicity Testing with *Strongylocentrotus purpuratus*

The short-term echinoderm sperm cell fertilization test consists of exposing purple sea urchin or sand dollar sperm to a series of effluent dilutions, after which the subsequent effects on successful fertilization of the eggs are determined. The specific procedures used in this test are described below.

The Lab Water Control medium for this test consisted of filtered (1 μm) seawater (collected from the UC Granite Canyon Marine Lab). The Lab Water Control medium and effluent sample were used to prepare test solutions at concentrations of 0.175, 0.35, 0.7, 1.4, and 2.8% effluent. Routine water quality characteristics (pH, D.O., and salinity) were measured for each test solution prior to use in this test.

Sperm and eggs were generated from gravid adult purple urchins, *S. purpuratus*. The gravid adult urchins were obtained from a commercial supplier (David Gutoff, San Diego, CA). Upon receipt at the PER lab, the urchins were held in tanks of aerated, filtered seawater at 12°C. Spawning of the urchins was induced by injection with 0.5 M KCl, followed by vigorous shaking of the animals to stimulate gamete release, as per EPA guidelines. The gametes from each spawning individual were collected and examined microscopically; the gametes exhibiting the best quality (as determined from morphology and trial fertilization) were pooled to provide a composite of high quality sperm and a composite of high quality eggs.

There were four replicates at each test treatment. Each test replicate consisted of a 30-mL glass vial to which 5 mL of appropriate test solution was added. The test was initiated with the inoculation of an appropriate quantity of sperm into each replicate vial to achieve a final sperm-to-egg ratio of 500:1. After a 20-min exposure period, ~1000 eggs were inoculated into each vial. After an additional 20-min exposure, the test was terminated with all of the test embryos being fixed by the addition of 1.0 mL of 5% glutaraldehyde.

The contents of each preserved test vial were subsequently examined microscopically to determine the percentage of embryos exhibiting complete fertilization. The resulting percentage fertilization data for each test treatment were analyzed in order to characterize any statistically significant reductions in successful fertilization that may have been caused by the effluent; determination of the key statistical endpoints were made using the CETIS® statistical software.

2.2.1 Reference Toxicant Testing of the Purple Urchin Embryos

In order to assess the sensitivity of the urchin sperm to toxicant stress, a reference toxicant test was performed concurrently with the effluent test. The reference toxicant test was performed similarly to the effluent test, but used test solutions consisting of Lab Water Control medium spiked with KCl at concentrations of 0.25, 0.5, 1, 2, and 4 g/L KCL. The resulting test response data were analyzed to determine key dose-response point estimates (e.g., EC_{50}); all statistical analyses were made using the CETIS® software. These response endpoints were then compared to the "typical response" range established by the mean ± 2 SD of the point estimates generated by the reference toxicant test database.

3. RESULTS

3.1 Effects of Anchorage Effluent on Purple Urchins

The results of this test are summarized below in Table 2. The normal embryo fertilization NOEC was 2.8% effluent, resulting in 35.7 TUc (where TUc = 100/NOEC). The test data and summary of statistical analyses for this test are presented in Appendix B.

Table 2. Effects of Anchorage effluent on echinoderm (purple urchin) sperm fertilization.

Effluent Treatment	Mean % Successful Fertilization
Lab Control (Filtered Seawater)	97.5
0.175%	99.0
0.35%	99.5
0.7%	99.3
1.4%	98.5
2.8%	98.3
Summary of Key Statistics	
NOEC =	2.8% effluent
TUc (where TUc = 100/NOEC) =	35.7
EC_{15} =	>2.8% effluent[a]
EC_{25} =	>2.8% effluent
EC_{40} =	>2.8% effluent
EC_{50} =	>2.8% effluent

a - Due to the absence of significant impairment, the EC point estimates could not be calculated, but can be determined by inspection to be >2.8% effluent.

3.1.1 Reference Toxicant Toxicity to the Purple Urchin

The results of this test are summarized below in Table 3. The EC50 for this test was consistent with the "typical response" range established by the reference toxicant database for this species, indicating that these organisms were responding to toxic stress in a typical and consistent fashion. The test data & summary of statistical analyses for this test are presented in Appendix C.

Table 3. Reference toxicant testing: Effects of KCl on echinoderm sperm fertilization.

KCl Treatment (g/L)	Mean % Successful Fertilization
Lab Control	99.8
0.25	98.3
0.5	**93.3***
1	**92.7***
2	**0***
4	**0***
Summary of Key Statistics	
EC50 =	1.30 g/L KCl

* - The response at this test treatment was significantly less than the Lab Control treatment response at $p < 0.05$.

4. SUMMARY AND CONCLUSIONS

Chronic Effects of Anchorage Effluent on Purple Urchin Sperm Fertilization
There were ***no*** significant reductions in successful fertilization at the effluent concentrations tested; the NOEC was 2.8% effluent, resulting in 35.7 TUc.

Purple Urchin Test Endpoint = Mean % Successful Fertilization	
NOEC = 2.8% effluent	TUc (= 100/NOEC) = 35.7 TUc

4.1 QA/QC Summary

Test Conditions – All test conditions (pH, D.O., temperature, etc.) were within acceptable limits. All analyses were performed according to laboratory Standard Operating Procedures.

Negative Control – The test organism responses at the Lab Control treatments were within acceptable limits.

Positive Control – The reference toxicant test results were consistent with the "typical response" ranges established by the reference toxicant test database, indicating that these test organisms were responding to toxic stress in a typical fashion.

Concentration Response Relationships – The concentration-response relationships for this these tests were evaluated as per EPA guidelines (EPA-821-B-00-004), and were determined to be acceptable.

Appendix A

Chain-of-Custody Record for the Collection and Delivery of the Anchorage Effluent Sample

KINNETIC LABORATORIES, INC. CHAIN OF CUSTODY RECORD

LABORATORY:	**FROM:**	
ATTN: Scott Ogle 707-207-7760 Pacific EcoRisk 2250 Cordelia Rd. Fairfield, CA 94534	Kinnetic Laboratories, Inc. 704 W. 2nd Ave. Anchorage, AK 99501 Attn: Mark Savoie/Gary Lawley 907.276.6178	
Required Completion Date: ASAP	P.O. #:AK14-1001	KLI Proj. #: 516.03

Static renewal chronic testing using 1) *Strongylocentruss purpuratus* (Purple urchin) fertilization test. Test following procedures in accordance with *Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Esturine Organisms*, (EPA/600/4-87/028). At least five dilutions must be tested, including a ZID boundary concentration of 0.70%, and two concentrations above (1.4 and 2.8%) and two concentrations below 0.70% (0.35 and 0.175). Reference toxicant testing must be performed concurrently.

	Preservative: None (4° C)	**Type of Container:** 1 gallon cubitainer

SAMPLE IDENTIFICATION #	NO. OF CONTAINERS	SAMPLE DATE	SAMPLE TIME	CONDITION UPON RECEIPT	ASSIGNED LABORATORY NUMBER
MOA14TOX004	1	6/11/14 0900			

DATA REPORTS **MUST** INCLUDE THE FOLLOWING: SAMPLE ID NUMBER, ANALYTICAL METHOD, DETECTION LIMIT, DATE OF EXTRACTION, DATE OF ANALYSIS, ANALYTICAL RESULTS, AND SIGNATURE OF QA REVIEWER.

Please return all completed original COCs to KLI Anchorage.

RELINQUISHED BY:	DATE AND TIME	TRANSPORTED BY:	RECEIVED BY:	DATE AND TIME
Alex W. Swirt	6/11/14 1139	~~FED EX~~ Hand		6/11/14 11:39
RELINQUISHED BY:	DATE AND TIME	TRANSPORTED BY:	RECEIVED BY:	DATE AND TIME
	6/11/14 11:55	FED EX	Mulay Allen MA	6/12/14 1050
RELINQUISHED BY:	DATE AND TIME	TRANSPORTED BY:	RECEIVED BY:	DATE AND TIME

SAMPLED BY (NAME/SIGNATURE): _____

Appendix B

Test Data and Summary of Statistics for the Evaluation of the Chronic Toxicity of Anchorage Effluent to Purple Urchin Sperm Fertilization

CETIS Summary Report

Echinoid Fertilization Test — **Pacific EcoRisk**

Batch ID:	01-0824-2857	**Test Type:**	Fertilization	**Analyst:**	Alison Briden
Start Date:	12 Jun-14 17:15	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Filtered Seawater
Ending Date:	16 Jun-14 17:55	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	4d 1h	**Source:**	Gutoff	**Age:**	N/A

Sample ID:	10-6049-2128	**Code:**	EFF	**Client:**	Kinnetic Labs
Sample Date:	11 Jun-14 09:00	**Material:**	Effluent	**Project:**	22580
Receive Date:	12 Jun-14 10:50	**Source:**	Kinnetic Laboratories, Inc.		
Sample Age:	32h (1.7 °C)	**Station:**	MOA14TOX004		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
02-0945-9842	Fertilization Rate	2.8	>2.8	NA	3.08%	35.71	Dunnett Multiple Comparison Test

Fertilization Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	4	0.975	0.973	0.977	0.97	0.98	0.00289	0.00577	0.59%	0.0%
0.175		4	0.99	0.987	0.993	0.98	1	0.00408	0.00817	0.83%	-1.54%
0.35		4	0.995	0.991	0.999	0.98	1	0.005	0.01	1.01%	-2.05%
0.7		4	0.993	0.989	0.996	0.98	1	0.00479	0.00957	0.97%	-1.79%
1.4		4	0.985	0.983	0.987	0.98	0.99	0.00289	0.00577	0.59%	-1.03%
2.8		4	0.983	0.975	0.99	0.96	1	0.0103	0.0206	2.1%	-0.77%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water Contr	0.97	0.98	0.98	0.97
0.175		0.99	0.99	1	0.98
0.35		1	0.98	1	1
0.7		0.99	1	1	0.98
1.4		0.99	0.98	0.98	0.99
2.8		0.96	0.97	1	1

Fertilization Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water Contr	97/100	98/100	98/100	97/100
0.175		99/100	99/100	100/100	98/100
0.35		100/100	98/100	100/100	100/100
0.7		99/100	100/100	100/100	98/100
1.4		99/100	98/100	98/100	99/100
2.8		96/100	97/100	100/100	100/100

CETIS Analytical Report

Echinoid Fertilization Test — Pacific EcoRisk

Analysis ID:	02-0946-9842	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.5
Analyzed:	16 Jun-14 15:49	Analysis: Parametric-Control vs Treatments	Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	3.08%	2.8	>2.8	NA	35.71

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Water Control		0.175	-1.83	2.41	0.079	6	0.9985	CDF	Non-Significant Effect
		0.35	-2.6	2.41	0.079	6	0.9999	CDF	Non-Significant Effect
		0.7	-2.21	2.41	0.079	6	0.9995	CDF	Non-Significant Effect
		1.4	-1.13	2.41	0.079	6	0.9879	CDF	Non-Significant Effect
		2.8	-1.19	2.41	0.079	6	0.9899	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01843812	0.003687623	5	1.72	0.1810	Non-Significant Effect
Error	0.03859296	0.002144054	18			
Total	0.05703108		23			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.77	15.1	0.2383	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.963	0.884	0.4917	Normal Distribution

Fertilization Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Water Contr	4	0.975	0.966	0.984	0.975	0.97	0.98	0.00289	0.59%	0.0%
0.175		4	0.99	0.977	1	0.99	0.98	1	0.00408	0.83%	-1.54%
0.35		4	0.995	0.979	1	1	0.98	1	0.005	1.01%	-2.05%
0.7		4	0.993	0.977	1	0.995	0.98	1	0.00479	0.97%	-1.79%
1.4		4	0.985	0.976	0.994	0.985	0.98	0.99	0.00289	0.59%	-1.03%
2.8		4	0.983	0.95	1	0.985	0.96	1	0.0103	2.1%	-0.77%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Water Cont	4	1.41	1.38	1.44	1.41	1.4	1.43	0.00929	1.32%	0.0%
0.175		4	1.47	1.41	1.53	1.47	1.43	1.52	0.0188	2.55%	-4.24%
0.35		4	1.5	1.42	1.57	1.52	1.43	1.52	0.023	3.07%	-6.02%
0.7		4	1.49	1.41	1.56	1.5	1.43	1.52	0.0222	2.99%	-5.13%
1.4		4	1.45	1.41	1.49	1.45	1.43	1.47	0.012	1.66%	-2.62%
2.8		4	1.45	1.32	1.58	1.46	1.37	1.52	0.0401	5.53%	-2.77%

Echinoderm Fertilization Toxicity Test Data Sheet

Client: **Kinnetic Anchorage**
Test Material: Effluent
Test Species: *S. purpuratus*
Test ID #: 57612
Project #: 22580
Sample Salinity adjusted with : –

Test Start Date: 6/12/14
Test End Date: 6/12/14
Enumeration Date: 6/13/14
Investigator: AB

Concentration	Replicate	Number of Fertilized Eggs	Number of Unfertilized Eggs	Total Number of Eggs	Percent Fertilization
Control	A	97	3	100	97
	B	98	2	100	98
	C	98	2	100	98
	D	97	3	100	97
0.175%	A	99	1	100	99
	B	99	1	100	99
	C	100	0	100	100
	D	98	2	100	98
0.35%	A	100	0	100	100
	B	98	2	100	98
	C	100	0	100	100
	D	100	0	100	100
0.7%	A	99	1	100	99
	B	100	0	100	100
	C	100	0	100	100
	D	98	2	100	98
1.4%	A	99	1	100	99
	B	98	2	100	98
	C	98	2	100	98
	D	99	1	100	99
2.8%	A	96	4	100	96
	B	97	3	100	97
	C	100	0	100	100
	D	100	0	100	100

Echinoderm Fertilization Toxicity Test Water Chemistry Data

Client: Kinnetic Anchorage
Test Material: Effluent
Test Species: *S. purpuratus*
Test ID#: 57612 Project #: 22580
Sample Salinity adjusted with: –

Organism Log#: 8301 Age: N/A
Organism Supplier: Gutoff
Control/Diluent: FSW
Test Date: 6/12/14 Randomization: –

Treatment	Temperature (°C)	pH	D.O. (mg/L)	Salinity (ppt)	Signoff
Control	12.5	7.73	8.7	33.0	Date: 6/12/14
0.175%	12.5	7.78	8.7	33.4	Sample ID: 35427
0.35%	12.5	7.78	8.7	33.3	Test Solution Prep: MF
0.7%	12.5	7.79	8.6	33.2	New WQ: SVV
1.4%	12.5	7.78	8.6	33.0	Innoculation Time: 1715
2.8%	12.5	7.77	8.7	32.6	Innoculation Signoff: MB
Meter ID	B2A	pH21	RD11	EC04	

Echinoderm Fertilization Toxicity Test Data Sheet

Client:	**Kinnetic Anchorage**	Test Start Date:	6/12/14
Test Material:	Effluent	Test End Date:	6/12/14
Test Species:	*S. purpuratus*	Enumeration Date:	6/13/14
Test ID #:	57612	Investigator:	KB
Project #:	22580	Effluent Salinity Adjusted with:	–

Treatment	Replicate	Number of Fertilized Eggs	Number of Unfertilized Eggs	Total Number of Eggs	Percent Fertilization
Lab Water Control	A	97	3	100	97
	B	98	2	100	98
	C	98	2	100	98
	D	97	3	100	97
Sperm Blank (eggs only) Negative Lab Water Control	A	0	100	100	0
	B	0	100	100	0
	C	0	100	100	0
	D	0	100	100	0
Sperm Blank (eggs only) Negative Effluent Control (11.2% Effluent) MF 2.8%	A	0	100	100	0
	B	0	100	100	0
	C	0	100	100	0
	D	0	100	100	0

Appendix C

Test Data and Summary of Statistics for the Reference Toxicant Evaluation of the Purple Urchin Sperm

CETIS Summary Report

Report Date: 16 Jun-14 15:33 (p 1 of 1)
Test Code: 57613 | 09-1969-9393

Echinoid Fertilization Test — Pacific EcoRisk

Batch ID:	01-0590-6021	Test Type:	Fertilization	Analyst:	Alison Briden
Start Date:	12 Jun-14 17:15	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Filtered Seawater
Ending Date:	12 Jun-14 17:55	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable
Duration:	40m	Source:	Gutoff	Age:	N/A

Sample ID:	17-4622-1108	Code:	KCl	Client:	Reference Toxicant
Sample Date:	12 Jun-14 17:15	Material:	Potassium chloride	Project:	22581
Receive Date:	12 Jun-14 17:15	Source:	Reference Toxicant		
Sample Age:	NA (12.5 °C)	Station:	In House		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
07-9213-4432	Fertilization Rate	0.25	0.5	0.3536	1.62%		Bonferroni Adj t Test

Point Estimate Summary

Analysis ID	Endpoint	Level	g/L	95% LCL	95% UCL	TU	Method
00-0015-0401	Fertilization Rate	EC50	1.3	1.27	1.34		Trimmed Spearman-Kärber

Fertilization Rate Summary

C-g/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Water Contr	4	0.998	0.996	0.999	0.99	1	0.0025	0.005	0.5%	0.0%
0.25		4	0.983	0.979	0.986	0.97	0.99	0.00479	0.00957	0.98%	1.5%
0.5		4	0.933	0.919	0.946	0.9	0.98	0.018	0.0359	3.85%	6.52%
1		3	0.927	0.922	0.931	0.92	0.94	0.00667	0.0115	1.25%	7.1%
2		3	0	0	0	0	0	0	0		100.0%
4		4	0	0	0	0	0	0	0		100.0%

Fertilization Rate Detail

C-g/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water Contr	1	1	1	0.99
0.25		0.99	0.98	0.99	0.97
0.5		0.98	0.94	0.91	0.9
1		0.92	0.94	0.92	
2		0	0	0	
4		0	0	0	0

Fertilization Rate Binomials

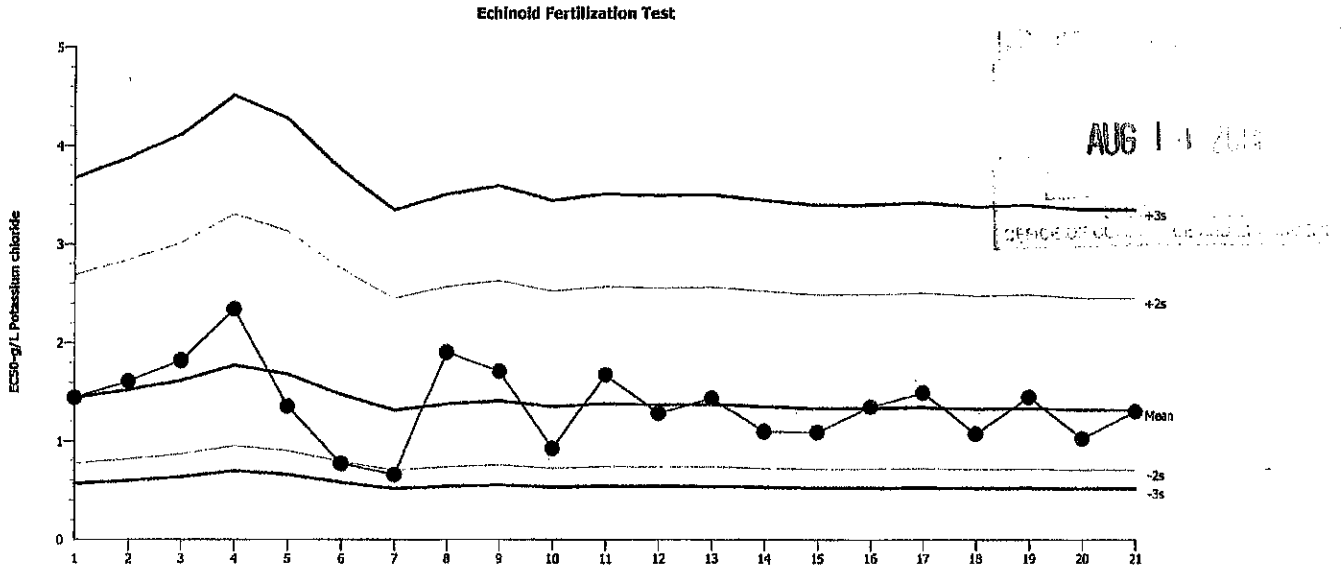
C-g/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Water Contr	100/100	100/100	100/100	99/100
0.25		99/100	98/100	99/100	97/100
0.5		98/100	94/100	91/100	90/100
1		92/100	94/100	92/100	
2		0/100	0/100	0/100	
4		0/100	0/100	0/100	0/100

CETIS QC Plot

Report Date: 16 Jun-14 15:39 (1 of 1)

Echinoid Fertilization Test — **Pacific EcoRisk**

Test Type:	Fertilization	**Organism:**	Strongylocentrotus purpuratus (Purpl	**Material:**	Potassium chloride
Protocol:	EPA/600/R-95/136 (1995)	**Endpoint:**	Fertilization Rate	**Source:**	Reference Toxicant-REF



Mean:	1.318	**Count:**	20	**-2s Warning Limit:**	0.7075	**-3s Action Limit:**	0.5185
Sigma:	NA	**CV:**	36.50%	**+2s Warning Limit:**	2.454	**+3s Action Limit:**	3.349

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2011	Dec	15	13:55	1.444	0.1269	0.2957			04-6051-4150	20-8134-1190
2	2012	Mar	8	16:22	1.61	0.2927	0.6453			05-0740-0748	15-5935-0686
3		Apr	6	15:30	1.823	0.5059	1.045			04-2265-9762	19-9125-4309
4			25	18:20	2.342	1.025	1.851			10-8393-5625	06-2730-7786
5		Aug	10	15:00	1.354	0.03623	0.08725			01-9226-6824	09-0663-0632
6		Oct	5	17:17	0.7751	-0.5425	-1.707			13-9975-3204	21-3214-1203
7			25	17:20	0.661	-0.6565	-2.218	(-)		15-6047-1276	05-8563-4140
8	2013	Jan	31	16:20	1.905	0.587	1.185			20-4482-2275	16-6771-7267
9		Apr	5	15:40	1.712	0.3942	0.8419			09-0614-8270	15-9800-9383
10		May	2	19:20	0.9268	-0.3908	-1.132			10-7105-5755	13-0792-4685
11			7	15:58	1.677	0.3595	0.776			00-1706-4139	06-3677-7130
12		Aug	28	17:02	1.284	-0.03339	-0.08255			06-2696-3137	00-3784-6188
13		Nov	6	14:36	1.438	0.1205	0.2815			14-3825-5642	11-2685-4555
14		Dec	5	15:50	1.101	-0.2167	-0.578			06-4350-3308	19-1664-9754
15	2014	Feb	7	14:20	1.09	-0.2274	-0.6094			11-6688-0585	16-9103-0015
16			10	18:00	1.348	0.03028	0.07307			02-9297-3328	14-6769-1689
17			18	15:47	1.496	0.1782	0.408			13-4044-9511	15-4518-0369
18			27	12:40	1.073	-0.2445	-0.6602			06-6744-5582	01-2061-9249
19			28	16:31	1.449	0.1314	0.3059			16-4152-6779	20-0611-0213
20		Apr	11	14:00	1.025	-0.2923	-0.8067			08-4721-0586	05-0420-5443
21		Jun	12	17:15	1.304	-0.01355	-0.03324			09-1969-9393	00-0015-0401

Echinoderm Fertilization Reference Toxicant Test Data Sheet

Client: **Reference Toxicant**
Test Material: Potassium Chloride
Test Species: *D. excentricus - S. purpuratus (circle)*
Test ID #: 57613
Project #: 22581

Test Start Date: 6/12/14
Test End Date: 6/12/14
Enumeration Date: 6/13/14
Investigator: AB

Concentration (g/L KCl)	Replicate	Number of Fertilized Eggs	Number of Unfertilized Eggs	Total Number of Eggs	Percent Normal Fertilization
Control	A	100	0	100	100
	B	100	0	100	100
	C	100	0	100	100
	D	99	1	100	99
0.25	A	99	1	100	99
	B	98	2	100	98
	C	99	1	100	99
	D	97	3	100	97
0.5	A	98	2	100	98
	B	94	6	100	94
	C	91	9	100	91
	D	90	10	100	90
1	A	92	8	100	92
	B	94	6	100	94
	C	30	70	100	30
	D	92	8	100	92
2	A	0	100	100	0
	B	0	100	100	0
	C	0	100	100	0
	D	81	19	100	81
4	A	0	100	100	0
	B	0	100	100	0
	C	0	100	100	0
	D	0	100	100	0

Echinoderm Fertilization Reference Toxicant Test Data Sheet

Client: **Reference Toxicant**
Test Material: Potassium Chloride
Test Species: *D. excentricus - S. purpuratus (circle)*
Test ID #: 57613
Project #: 22581

Test Start Date: 6/12/14
Test End Date: 6/12/14
Enumeration Date: 6/13/14
Investigator: XB

Treatment	Replicate	Number of Fertilized Eggs	Number of Unfertilized Eggs	Total Number of Eggs	Percent Fertilization
Lab Control (Natural Sea Water)	A	100	0	100	100
	B	100	0	100	100
	C	100	0	100	100
	D	99	1	100	99
Sperm Blank (eggs only) Lab Water Control	A	0	100	100	0
	B	0	100	100	0
	C	0	100	100	0
	D	0	100	100	0
Sperm Blank (eggs only) 4 g/L Control	A	0	100	100	0
	B	0	100	100	0
	C	0	100	100	0
	D	0	100	100	0
	A				
	B				
	C				
	D				

Echinoderm Fertilization Reference Toxicant Test Water Chemistry Data

Client: **Reference Toxicant**
Test Material: Potassium Chloride
Test Species: *D. excentricus - S. purpuratus (circle)*
Test ID#: 57613 Project #: 22581

Organism Log#: 8301 Age: N/A
Organism Supplier: Gutoff
Control/Diluent: FSW
Test Date: 6/12/14 Randomization: -

Treatment (g/L KCl)	Temperature (°C)	pH	D.O. (mg/L)	Salinity (ppt)	Signoff
Control	12.5	~~7.80~~ 7.77 SW	9.0	33.1	Date: 6/12/14
0.25	12.5	~~7.80~~ 7.77 SW	9.0	MF ~~33.9~~ 33.6	Test Solution Prep: MF
0.5	12.5	7.68	9.0	33.9	New WQ: SW
1	12.5	7.73	8.8	34.6	Innoculation Time: 1715
2	12.5	7.74	8.7	35.7	Innoculation Signoff: KB
4	12.5	7.75	8.7	37.7	
Meter ID	82 A	pH 21 SW	RD11	EC04